film. Then, 200 µl of the Substrate Buffer is added and allowed to soak in.

The assay membrane is placed in a camera luminometer device equipped with pre-exposed calibration scales for HSV, HPV and CMV.

The chemiluminescent light emission generated as a function of the enzymes -- alkaline phosphatase, carboxyl esterase and β-galactosidase -- is imaged through a mask containing three narrow bandpass Kodak Wrattan gelatin filters (approximately 1cm in diameter), which isolate the blue emission from the phenyl phosphate dioxetane, the cyan emission from the phosphoryloxytrifluoromethylbenzopyranyl dioxetane and the green emnission from the galactosyloxybenzopyranyl dioxetane, respectively.

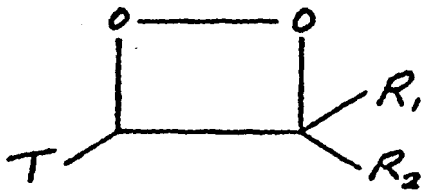
The relative levels of HSV, HPV and CMV present in the sample are determined by a comparison of the appropriate image brightness with the relevant calibration scale.

The above discussion of this invention is directed primarily to preferred embodiments and practices thereof. It will be readily apparent to those skilled in the art that further changes and modifications in the actual implementation of the concepts described herein can easily be made without departing from the spirit and scope of the invention as defined by the following claims.

WE CLAIM:

1. A process in which light of different wavelengths is simultaneously released from two or more enzymatically decomposable chemiluminescent 1,2-dioxetane compounds, said compounds being configured, by means of the inclusion of a different light emitting fluorophore in each of them, to each emit light of said different wavelengths, which comprises decomposing each of said compounds by means of a different enzyme.

2. The process of claim 1 in which each of said compounds is represented by the general formula:



wherein R_1 represents hydrogen, or a bond when R_2 represents a substituent bonded to the dioxetane ring through a spiro linkage, or an organic substituent that does not interfere with the production of light and that satisfies the valence of the dioxetane ring carbon atom to which it is attached, or a light-emitting fluorophore-forming fluorescent chromophore group bonded to the dioxetane ring through a single bond or a spiro linkage, to which an enzyme-cleavable group is bonded, R_2 represents hydrogen, or a bond when R_1 represents a substituent bonded to the dioxetane ring through a spiro linkage, or a light-emitting

fluorophore-forming fluorescent chromophore group bonded to the dioxetane ring through a single bond or a spiro linkage, to which an enzyme-cleavable group is bonded, at least one of R_1 and R_2 being such light-emitting fluorophore-forming fluorescent chromophore group, and T represents a stabilizing group that prevents the dioxetane compound from decomposing before the bond in the enzyme-cleavable group is intentionally cleaved.

3. The process of claim 2 in which the process carried out is a step in an immunoassay.

4. The process of claim 3 in which the immunoassay is for the detection of specific binding pairs comprising an antigen and an antibody.

5. The process of claim 3 in which the labels used in the assay are enzymes.

6. The process of claim 3 in which the labels used in the assay are the chemiluminescent 1,2-dioxetane compounds.

7. The process of claim 3 in which the immunoassay is for the detection of an enzyme.

8. The process of claim 3 in which the immunoassay is for the detection of hormones.

9. The process of claim 2 in which the process carried out is a step in a chemical assay.

10. The process of claim 9 in which the chemical assay is for the detection of chemical substances which, during the assay, are caused to decompose to form substances capable of causing the chemiluminescent 1,2-dioxetane compounds to decompose.

11. The process of claim 10 in which one of the chemical substances is glucose.

12. The process of claim 10 in which one of the chemical substances is cholesterol.

13. The process of claim 2 in which the process carried out is a nucleic acid probe assay.

14. The process of claim 13 in which the nucleic acid probe assay is for the detection of viruses.

15. The process of claim 2 in which the process carried out is a histocompatibility assay.

16. The process of claim 2 in which the process carried out is a technique for studying the microstructure of a macromolecule.

17. The process of claim 2 in which the process carried out is a multi-channel assay carried out in the presence of at least two of the chemiluminescent 1,2-dioxetane compounds as recited in claim 2 as substrates, each of which upon decomposition emits light of a different wavelength from the other(s) and each of which has a labile ring substituent cleavable by a different means from the other(s).

18. The process of claim 2 in which the process carried out is a multi-channel assay carried out in the presence of at least one of the chemiluminescent 1,2-dioxetane compounds as recited in claim and at least one other chemically decomposable chemiluminescent compound as substrates, each of which chemiluminescent 1,2-dioxetane compounds and other chemiluminescent compounds emit light of a different wavelength from the other(s).